Matter No.: 24601-402P Sheet 1 of 5
Applicant(s): Hadlaczky et al.
Entitled: ARTIFICIAL CHROMOSOMES, USES
THEREOF AND METHODS FOR PREPARING
ARTIFICIAL CHROMOSOMES

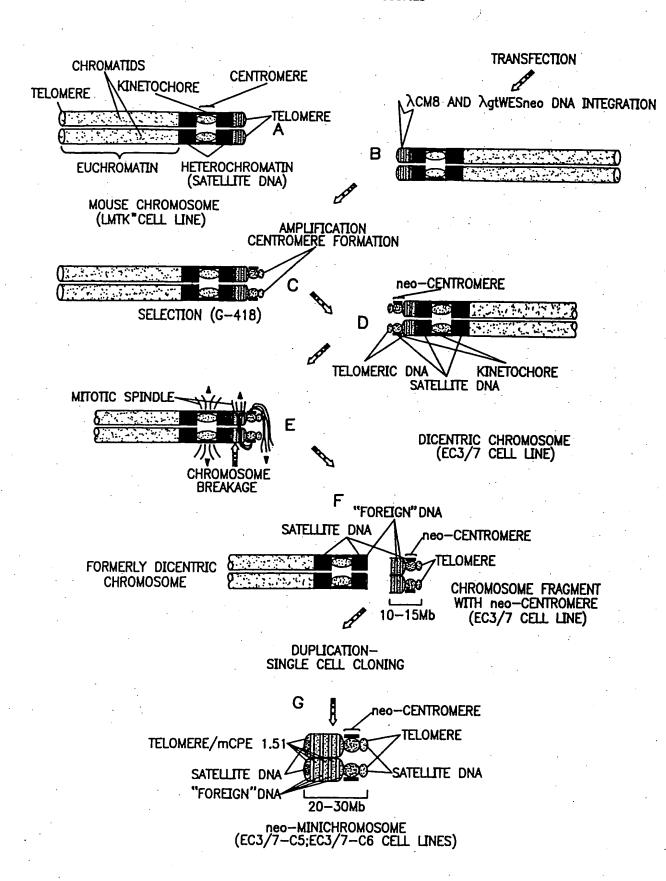


FIG. I

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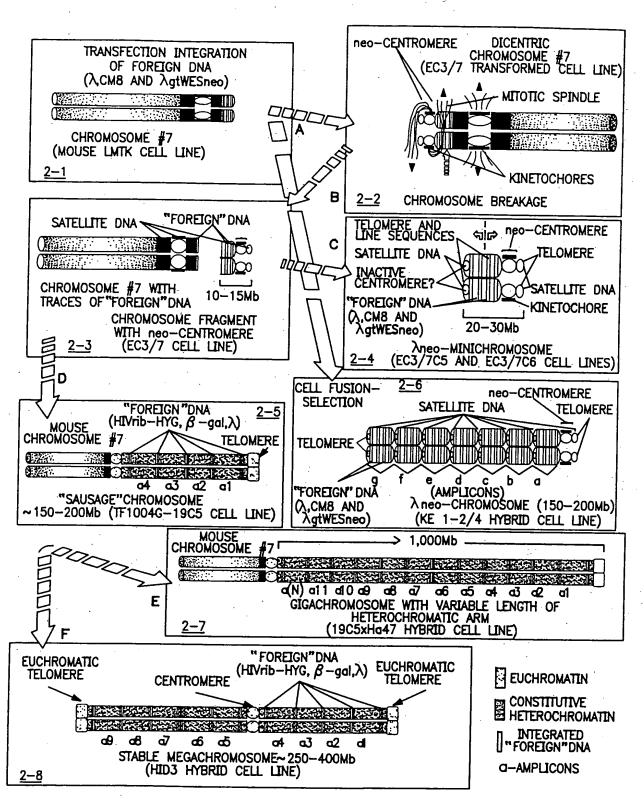


FIG. 2

Matter No.: 24601-402P Sheet 3 of 5
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PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR) ECONDARY ORIGINS OF REPLICATION MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO-7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES NTEGRATION OF "FOREIGN"DNA (pH132, pCH110,λ) REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA **CHROMATIDS** ~7.5Mb ~ 15Mb ~30 Mb **AMPLICON**

STABLE MEGACHROMOSOME (~250-400Mb)

TELOMERE

FIG. 3

CENTROMERE

TELOMERE

EC3/7

MOUSE LMTK" (FIBROBLAST CELL LINE WITH neo-CENTROMERE)

SINGLE-CELL SUBCLONING

FUSION WITH CHO K20 CELLS AND SELECTION WITH G418 AND HAT

EC3/7C5

MOUSE LMTK FIBROBLAST CELL LINE WITH THE neo-MINICHROMOSOME AND THE FORMERLY DICENTRIC **CHROMOSOME**

KE1 - 2/4

MOUSE-HAMSTER HYBRID CELL LINE WITH THE STABLE Aneo-CHROMOSOME

COTRANSFECTION WITH PLASMIDS pH132 (ANTI-HIV RIBOZYME AND HYGROMYCIN-RESISTANCE GENES), pCH110 (lacZ GENE) AND λcl 875 Sam7 (λPHAGE), SELECTION WITH HYGROMYCIN B

TF1004G-19C5

MOUSE LMTK FIBROBLAST CELL LINE WITH neo-MINICHROMSOME AND STABLE SAUSAGE CHROMOSOME FUSION WITH CHINESE HAMSTER OVARY CELLS (CHO K20 CELL LINE), SELECTION WITH HAT AND HYGROMYCIN B.

19C5xHa4, 19C5xHa3 -- RECLONING -- 19C5xHa47 (CARRIES THE GIGACHROMSOME)

MOUSE-HAMSTER HYBRID CELL LINES CARRYING THE neo-MINICHROMOSOME AND THE SAUSAGE CHROMOSOME AND COUNTAINING A COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME

BrdU TREATMENT, SINGLE-CELL CLONING. SELECTION WITH HYGROMYCIN B

BrdU TREATMENT, SINGLE-CELL CLONING, SELECTION WITH G418, BrdU TREATMENT AND RECLONING

<u>H</u>1D3

MOUSE-HAMSTER HYBRID CELL LINE CARRYING A MEGACHROMOSOME BUT NO MINICHROMOSOME

> FUSION WITH CD4+ HeLa CELLS CONTAINING neor. SELECTION WITH G418 AND HYGROMYCIN B

G3D5

G3D6

IN G418

MOUSE-HAMSTER HYBRID CELL LINES CARRYING: MEGACHROMOSOME neo- MINICHROMOSOME ONLY

AND neo-

MINICHROMOSOME RECLONE AND GROW

IN G418 AND ▼ HYGROMYCIN B

GHB42

CARRIES MEGA-- CHROMOSOME .AND neo-MINICHROMOSOME

GB43

CARRIES neo-MINICHROMOSOME ONLY

RECLONE AND GROW

H1xHe41

MOUSE-HAMSTER-HUMAN HYBRID CELL LINE CARRYING THE MEGACHROMOSOME AND A SINGLE HUMAN

CHROMOSOME WITH CD4 AND

neor GENES; CONTAINS COMPLETE HAMSTER AND PARTIAL MOUSE GENOMES

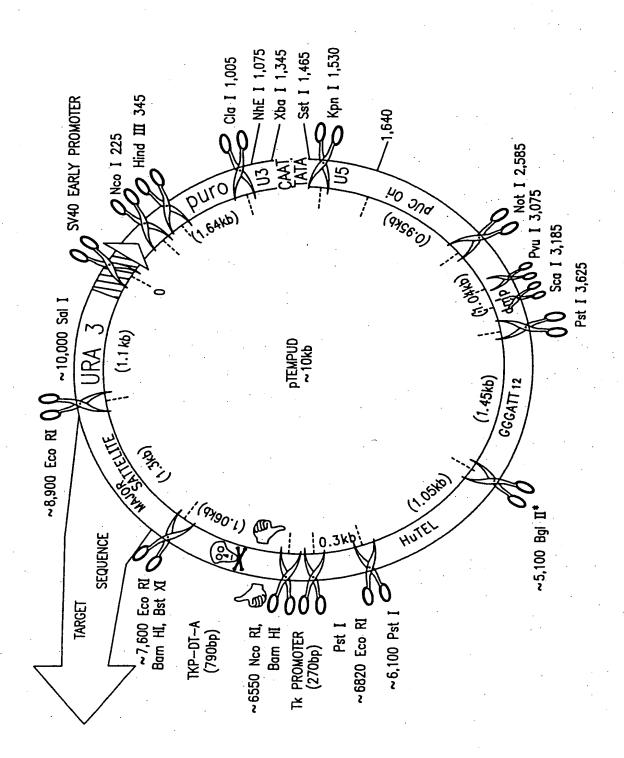


FIG. 5